

**What is claimed is:**

1. A method of staining bacteria comprising: working a polymethine dye on a sample in the presence of a substance capable of reducing nitrite ions to stain bacteria in the sample.

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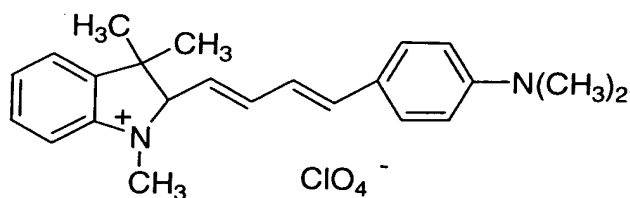
2. A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, asparatic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

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3. A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

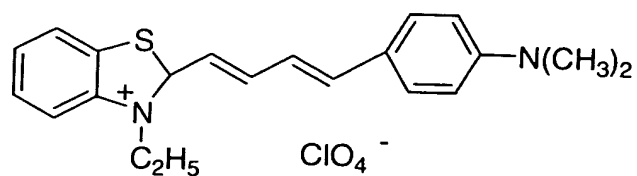
(1) Thiazole Orange;

(2)

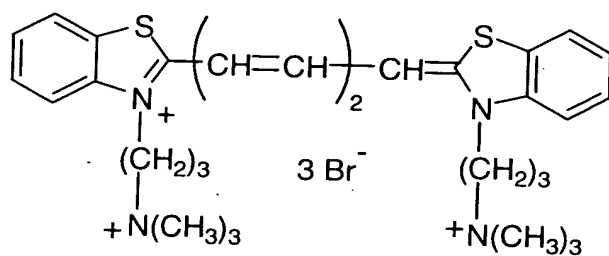


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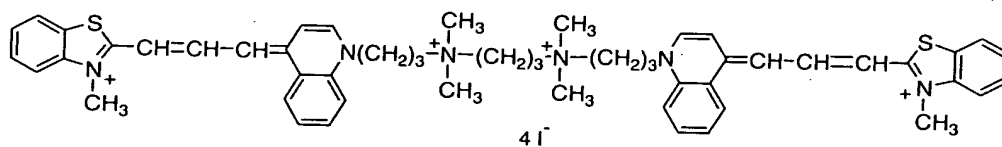
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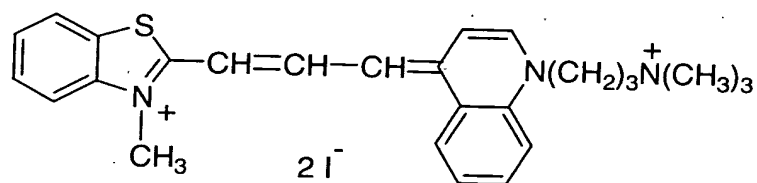
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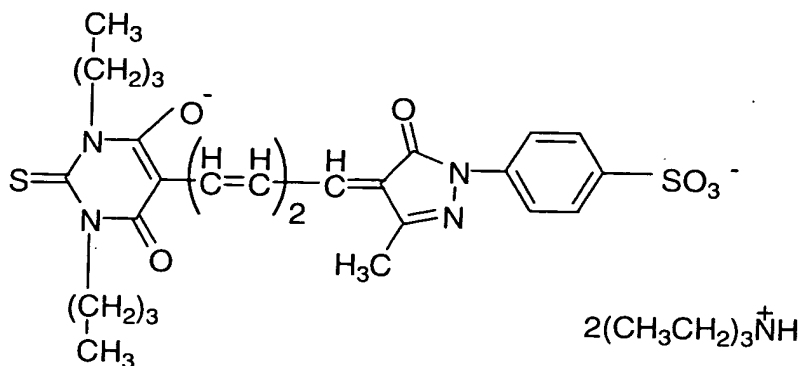
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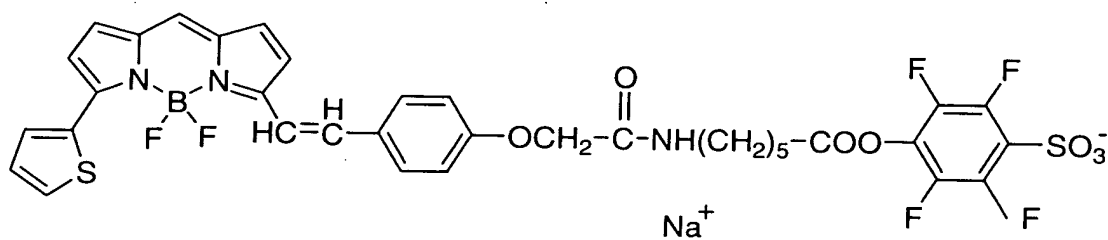
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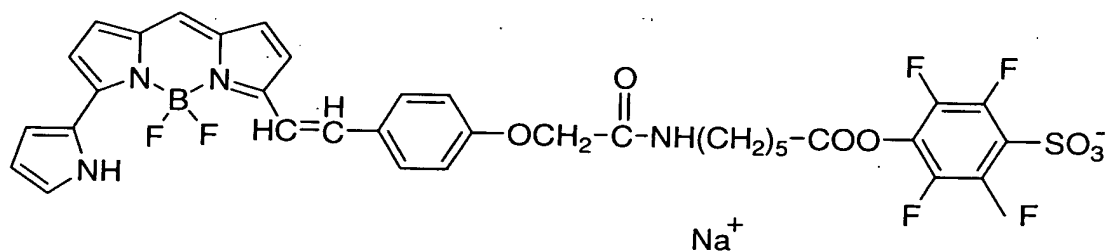


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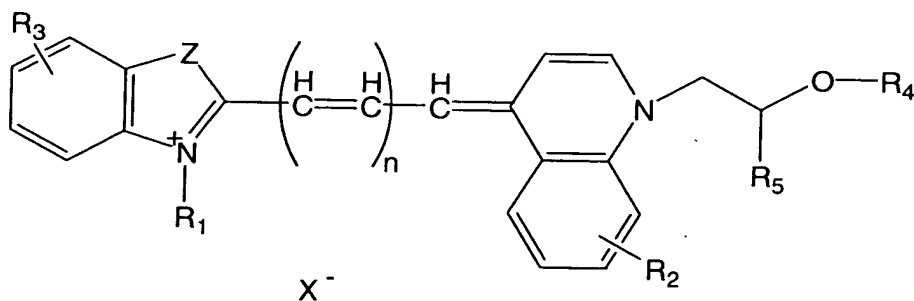


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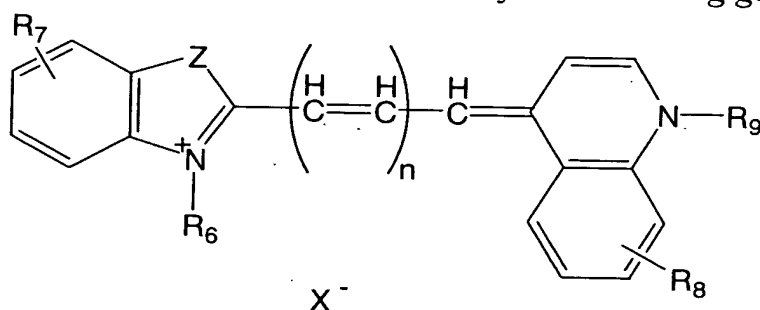


(10) a compound represented by the following general formula:



wherein  $R_1$  is a hydrogen atom or a  $C_{1-3}$  alkyl group;  $R_2$  and  $R_3$  are a hydrogen atom, a  $C_{1-3}$  alkyl group or a  $C_{1-3}$  alkoxy group;  $R_4$  is a hydrogen atom, an acyl group or a  $C_{1-3}$  alkyl group;  $R_5$  is a hydrogen atom or a  $C_{1-3}$  alkyl group which may be substituted;  $Z$  is a sulfur atom, an oxygen atom or a carbon atom substituted with a  $C_{1-3}$  alkyl group;  $n$  is 1 or 2;  $X^-$  is an anion; and

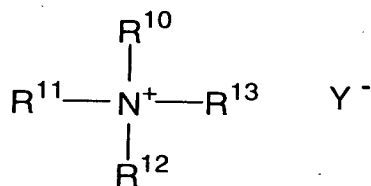
(11) a compound represented by the following general formula:



wherein  $R_1$  is a hydrogen atom or a  $C_{1-18}$  alkyl group;  $R_2$  and  $R_3$  are a hydrogen atom, a  $C_{1-3}$  alkyl group or a  $C_{1-3}$  alkoxy group;  $R_4$  is a hydrogen atom, an acyl group or a  $C_{1-18}$  alkyl group;  $Z$  is sulfur, oxygen or carbon having a  $C_{1-3}$  alkyl group;  $n$  is 0, 1 or 2;  $X^-$  is an anion.

4. A method according to claim 1, wherein the working is carried out in the existence with a cationic surfactant.

5. A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein  $R^{10}$  is a  $C_{6-18}$  alkyl group or  $(C_6H_5)-CH_2-$ ;  $R^{11}$ ,  $R^{12}$  and  
5  $R^{13}$ , the same or different, are a  $C_{1-3}$  alkyl group or a benzyl group;  $Y^{-}$   
is a halogen ion.

6. A method according to claim 5, wherein the quaternary  
ammonium salt is at least one selected from the group consisting of:  
10 decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt,  
tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium  
salt and octadecyl trimethyl ammonium salt.

7. A method according to claim 1, wherein the dye is worked  
15 under an acidic state.

8. A method according to claim 7, wherein the acidic state is set  
at pH 2.0-4.5.

20 9. A method according to claim 1, wherein a buffer of pKa 1-5.5 is  
used to maintain pH.

10. A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH,  $\epsilon$ -aminocaproic acid-HCl, fumaric acid-HCl,  $\beta$ -alanine-NaOH and glycine-NaOH.

11. A method according to claim 1, wherein the working is carried out in the existence with an inorganic salt of either sulfate or nitrate.

12. A method according to claim 1, wherein the dye is worked at 0.1 to 100 ppm in the sample.

13. A method according to claim 1, wherein the substance capable of reducing nitrite ions exists in the sample in such an amount that it can reduces the nitrite ions produced by bacteria of  $10^5$  to  $10^8$ /ml.

14. A method according to claim 1, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. A method according to claim 10, wherein the acid or the compound maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. A method of detecting and counting bacteria comprising the  
5 following steps of:

(1) working a polymethine dye on a sample by a method as described in any one of claims 1 to stain bacteria in the sample,

(2) introducing the thus treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by  
10 one with light to measure scattered light and fluorescent light emitted from each of the cells; and

(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the  
15 length of particles to count the number of the bacteria.

18. A method according to claim 17, wherein the step (1) is carried out by the steps of

(a) mixing a sample with an aqueous solution containing a  
20 substance capable of reducing nitrite ions and/or a cationic surfactant to accelerate dye transmissivity of bacteria;

(b) staining the bacteria for a predetermined period with a polymethine dye;

25 19. A method according to claim 17, wherein the step (3) of

discriminating and counting the bacteria is carried out in accordance with at least one selected from the following combinations of:

(i) a forward scattered light intensity and a forward scattered light pulse width;

5 (ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.

10 20. A diluent for bacterial stain comprising:  
a buffer for maintaining acidity; and  
an effective amount of a substance capable of reducing nitrite ions.

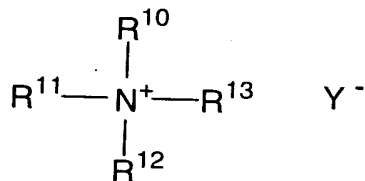
15 21. A diluent according to claim 20, wherein the substance capable of reducing nitrite ions is selected from the group consisting of:  
ascorbic acid, isoascorbic acid, aminomethanesulfonic acid,  
aminoethanesulfonic acid, glutamic acid, asparatic acid, Sp  
mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid,  
20 sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid,  
glycine, glutamine, asparagine, methionine, glutathione, cysteine,  
hydroxylamine and salts thereof; sulfanilamide; aminomethane;  
mercaptoethanol; thiophenol and urea.

25 22. A diluent according to claim 20 further comprising a cationic



surfactant.

23. A diluent according to claim 22, wherein the cationic surfactant is a quaternary ammonium salt represented by the



- 5 following formula:

wherein  $\text{R}^{10}$  is a  $\text{C}_{6-18}$  alkyl group or  $(\text{C}_6\text{H}_5)\text{-CH}_2\text{-}$ ;  $\text{R}^{11}$ ,  $\text{R}^{12}$  and  $\text{R}^{13}$ , the same or different, are a  $\text{C}_{1-3}$  alkyl group or a benzyl group;  $\text{Y}^{-}$  is a halogen ion.

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